Appl. No. 10/686,884 Amdt. dated March 9, 2005 Reply to Office Action of September 9, 2004

## **Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings of claims in the application:

## **Listing of Claims:**

1

2

3

1

2

8

1 (cancelled)

- 2 (currently amended) The material according to claim 5, wherein said linking group R<sup>14</sup> is a member selected from the group consisting of substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl and substituted or unsubstituted aryl.
  - 3-4 (cancelled)
  - 5 (currently amended): A material having the structure:

O 
$$R^1$$
 NHC(O)AA $^1$ —AA $^2$ —(AA $^1$ )<sub>J-2</sub>  $R^3$ 

3 wherein:

R<sup>1</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and R<sup>6</sup> are members independently selected from the group consisting of H, halogen, -NO<sub>2</sub>, -CN, -C(O)<sub>m</sub>R<sup>7</sup>, -C(O)NR<sup>8</sup>R<sup>9</sup>, -S(O)<sub>t</sub>R<sup>10</sup>, -SO<sub>2</sub>NR<sup>11</sup>R<sup>12</sup>, -OR<sup>13</sup>, substituted or unsubstituted alkyl and -R<sup>14</sup>-SS, with the proviso that at least one of R<sup>1</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and R<sup>6</sup> is -R<sup>14</sup>-SS;

wherein:

R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup>, R<sup>10</sup>, R<sup>11</sup>, R<sup>12</sup> and R<sup>13</sup> are members independently selected from the group consisting of H, substituted or unsubstituted alkyl and substituted or unsubstituted aryl;

12	R <sup>14</sup> is a linking group adjoining said fluorogenic moiety and said solid
13	support;
14	m is a member selected from the group consisting of the integers 1 and 2;
15	t is a member selected from the group consisting of the integers from 0 to
16	2; and
17	SS is a solid support;
18	$AA^{1}$ - $AA^{2}$ - $(AA^{i})_{J-2}$ is a peptide sequence, wherein each of $AA^{1}$ through $AA^{i}$ is an
19	amino acid residue which is a member independently selected from the
20	group of natural amino acid residues, unnatural amino acid residues and
21	modified amino acid residues;
22	J denotes the number of amino acid residues forming said peptide
23	sequence and is a member selected from the group consisting of
24	the numbers from 2 to 10, such that $J$ -2 is the number of amino
25	acid residues in the peptide sequence exclusive of AA1-AA2; and
26	$i$ denotes the position of said amino acid residue relevant to $AA^1$ and when
27	J is greater than 2, $i$ is a member selected from the group
28	consisting of the numbers from 3 to 10.

6 (currently amended): The material according to claim 5, having the structure:

$$\begin{array}{c|c}
O & & & O \\
O & & & & \\
C & & & & \\
R^4 & & & & \\
Z & & & & \\
SS & & & & \\
\end{array}$$

$$\begin{array}{c|c}
R^1 & O \\
O & & & \\
AA^1 - AA^2 - (AA^i)_{J-2} \\
R^3 & & & \\
Z & & & \\
SS & & & \\
\end{array}$$

2 3

5

1

wherein: Z is a member selected from the group consisting of -O-, and --NR<sup>16</sup>-, wherein R<sup>16</sup> is H, substituted or unsubstituted alkyl and substituted or unsubstituted aryl, and

6 c is a member selected from the integers from 0 to 6.

7 (currently amended): A material according to claim 6, having the structure:

1

2

3

4

5

6

7

8

1

2

1

2

- 8 (currently amended): A method of assaying for the presence of an enzymatically active protease in a sample, said method comprising:
  - (a) contacting said sample with a material according to claim 5 in such a manner whereby said fluorogenic moiety is released from said peptide sequence upon action of said protease, thereby producing a fluorescent moiety; and
  - (b) observing whether said sample undergoes a detectable change in fluorescence, said detectable change being an indication of the presence of said enzymatically active protease in said sample.
- 9 (original): The method according to claim 8, wherein said protease is a member selected from the group consisting of aspartic protease, cysteine protease, metalloprotease and serine protease.
  - 10 (original): The method according to claim 8, wherein said protease is a protease of a microorganism.
  - 11 (original): The method according to claim 10, wherein said microorganism is a member selected from the group consisting of bacteria, fungi, yeast, viruses, and protozoa.
- 1 12 (original): The method according to claim 8, wherein said sample is a clinical 2 sample from a subject.

I	13 (original): The method according to claim 8, further comprising (c)
2	quantifying said fluorescent moiety, thereby quantifying said protease.
1	14 (currently amended): A method of assaying for the presence of a selected
2	microorganism in a sample by probing the sequence specificity of peptide cleavage by a protease
3	of said microorganism, said method comprising:
4	(a) contacting a sample suspected of containing said selected microorganism with
5	a material according to claim 5, wherein said peptide comprises a
6	sequence that is selectively cleaved by said protease of said selected
7	microorganism, thereby releasing the fluorogenic moiety from the peptide
8	sequence;
9	(b) detecting the cleavage by detecting fluorescence arising from a fluorescent
10	moiety produced by cleavage of said fluorogenic moiety from said peptide
11	sequence, thereby confirming said presence of said selected
12	microorganism in said sample.
1	15 (original): The method according to claim 14, further comprising (c)
2	quantifying said fluorescence, thereby quantifying said protease of said microorganism.
	16 (cancelled)
1	17 (currently amended): The fluorogenic peptide according to claim 18, wherein
2	Y is an organic functional group selected from the group consisting of -COOR <sup>17</sup> , CONR <sup>17</sup> R <sup>21</sup> ,
3	$-C(O)R^{17}R^{21}$ , $-OR^{17}$ , $-SR^{17}$ , $-C(O)SR^{17}$ and $-NR^{17}R^{21}$
4	wherein, R <sup>17</sup> and R <sup>21</sup> are members independently selected from H, substituted or
5	unsubstituted alkyl and substituted or unsubstituted aryl.
1	18 (currently amended): A fluorogenic peptide having the structure:

O 
$$R^1$$
 NHC(O)AA $^1$ —AA $^2$ —(AA $^i$ )<sub>J-2</sub>
 $R^6$   $R^3$ 

2

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

3

wherein: R<sup>1</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and R<sup>6</sup> are members independently selected from the group consisting of H, halogen, -NO<sub>2</sub>, -CN, -C(O)<sub>m</sub>R<sup>6</sup>, -C(O)NR<sup>7</sup>R<sup>8</sup>, -S(O)<sub>t</sub>R<sup>9</sup>, -SO<sub>2</sub>NR<sup>10</sup>R<sup>11</sup>, -OR<sup>12</sup>, substituted or unsubstituted alkyl, -NHC(O)-P, and - $R^{20}$ -Y, with the proviso that at least one of  $R^1$ ,  $R^3$ ,  $R^4$ ,  $R^5$  and  $R^6$  is  $-R^{20}$ -Y, wherein: R<sup>6</sup>', R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup>, R<sup>10</sup>, R<sup>11</sup> and R<sup>12</sup> are members independently selected from the group consisting of H, substituted or unsubstituted alkyl and substituted or unsubstituted aryl; R<sup>20</sup> is either present or absent and is a member selected from the group consisting of substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl; Y is a member selected from the group consisting of organic functional groups and methyl; m is a member selected from the group consisting of the integers 1 and 2; and t is a member selected from the group consisting of the integers from 0 to 2.  $AA^{1}-AA^{2}-(AA^{i})_{L2}$  is a peptide sequence, wherein each of  $AA^{1}$  through  $AA^{i}$  is an amino acid residue which is a member independently selected from the group of natural amino acid residues, unnatural amino acid residues and

modified amino acid residues;

J denotes the number of amino acid residues forming said peptide

sequence and is a member selected from the group consisting of

the numbers from 2 to 10, such that J-2 is the number of amino

acid residues in the peptide sequence exclusive of AA<sup>1</sup>-AA<sup>2</sup>; and

i denotes the position of said amino acid residue in sequence relative to

AA<sup>1</sup> and when J is greater than 2, i is a member selected from the

group consisting of the numbers from 3 to 10.

19 (original): A fluorogenic peptide according to claim 18, having the structure:

$$O \longrightarrow O \longrightarrow NHC(O)AA^1 \longrightarrow AA^2 \longrightarrow (AA^1)_{J-2}$$

$$R^3$$

3 wherein:

1

2

1

c is a member selected from the group consisting of the integers from 0 to 6.

20 (original): A fluorogenic peptide according to claim 19, having the structure:

2

1

2

3

4

21 (original): The fluorogenic peptide according to claim 18, wherein said peptide sequence comprises a peptide bond that is cleaved by a protease releasing said fluorogenic moiety from said peptide sequence, thereby producing a fluorescent moiety and a peptide moiety.

1	22 (original). The hadrogenic peptide according to claim 21, wherein said
2	peptide bond is formed between a carboxyl of the carboxy-terminus amino acid residue and an
3	amine group of said fluorogenic moiety.
1	23 (currently amended): A method of assaying for the presence of an
2	enzymatically active protease in a sample, said method comprising:
3	(a) contacting a sample suspected of containing said protease with a peptide
4	according to claim 18 in such a manner whereby said fluorogenic moiety is released from said
5	peptide sequence upon action of said protease, thereby producing a fluorescent moiety; and
6	(b) observing whether said sample undergoes a detectable change in fluorescence
7	said detectable change being an indication of the presence of said enzymatically active protease
8	in said sample.
1	24 (original): The method according to claim 23, wherein said protease is a
2	member selected from the group consisting of aspartic protease, cysteine protease,
3	metalloprotease and serine protease.
1	25 (original): The method according to claim 23, wherein said protease is a
2	protease of a microorganism.
1	26 (original): The method according to claim 25, wherein said microorganism is
2	a member selected from the group consisting of bacteria, fungi, yeast, viruses, and protozoa.
1	27 (original): The method according to claim 23, wherein said sample is a
2	clinical sample from a subject.
1	28 (original): The method according to claim 27, wherein said subject is a
2	human.

1	29 (original): The method according to claim 23, further comprising (c)
2	quantifying said fluorescent moiety, thereby quantifying said protease.
1	30 (currently amended): A method of assaying for the presence of a selected
2	microorganism in a sample by probing the sequence specificity of peptide cleavage by a protease
3	of said microorganism, said method comprising:
4	(a) contacting a sample suspected of containing said selected microorganism with
5	a material according to claim 18, wherein said peptide comprises a
6	sequence that is selectively cleaved by a protease of a selected
7	microorganism, thereby releasing said fluorogenic moiety from said
8	peptide sequence;
9	(b) detecting said cleavage by detecting fluorescence arising from a fluorescent
10	moiety produced by cleavage of said fluorogenic moiety from said peptide
11	sequence, thereby confirming said presence of said selected
12	microorganism in said sample.
1	31 (original): The method according to claim 30, further comprising (c)
2	quantifying said fluorescence, thereby quantifying said protease of said microorganism.
1	32 (cancelled)
	33 (currently amended): The library according to claim 35, wherein said linking
	group R <sup>14</sup> is a member selected from the group consisting of substituted or unsubstituted alkyl,
	substituted or unsubstituted heteroalkyl and substituted or unsubstituted aryl.
1	34 (currently amended): The library according to claim 35, wherein Y is an
2	organic functional group selected from the group consisting of -COOR <sup>17</sup> , CONR <sup>17</sup> R <sup>21</sup> ,
3	$-C(O)R^{17}R^{21}$ , $-OR^{17}$ , $-SR^{17}$ , $-C(O)SR^{17}$ , and $-NR^{17}R^{21}$
4	wherein, R <sup>17</sup> and R <sup>21</sup> are members independently selected from H, substituted or
5	unsubstituted alkyl and substituted or unsubstituted aryl.

35 (currently amended): A library of fluorogenic peptides comprising at least a first peptide having a first peptide sequence covalently attached to a first fluorogenic moiety and a second peptide having a second peptide sequence covalently attached to a second fluorogenic moiety, said first peptide and said second peptide having the structure:

6 wherein:

R<sup>1</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> are members independently selected from the group consisting of H, halogen, -NO<sub>2</sub>, -CN, -C(O)<sub>m</sub>R<sup>7</sup>, -C(O)NR<sup>8</sup>R<sup>9</sup>, -S(O)<sub>t</sub>R<sup>10</sup>, -SO<sub>2</sub>NR<sup>11</sup>R<sup>12</sup>, -OR<sup>13</sup>, substituted or unsubstituted alkyl, -NH-C(O)-P, R<sup>20</sup>-Y and -R<sup>14</sup>-SS, with the proviso that for each peptide at least one of R<sup>1</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and R<sup>6</sup> is a member independently selected from -R<sup>14</sup>-SS and R<sup>20</sup>-Y,

wherein:

R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup>, R<sup>10</sup>, R<sup>11</sup>, R<sup>12</sup> and R<sup>13</sup> are members independently selected from the group consisting of H, substituted or unsubstituted alkyl and substituted or unsubstituted aryl;

R<sup>14</sup> is a linking group adjoining said fluorogenic moiety and the solid support;

R<sup>20</sup> is either present or absent and is a member selected from the group consisting of substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl;

Y is a member selected from the group consisting of organic functional groups and methyl;

24 m is a member selected from the group consisting of the integers from 1 to 25 2; 26 t is a member selected from the group consisting of the integers from 0 to 27 2; and SS is a solid support; 28  $AA^{1}-AA^{2}-(AA^{i})_{L2}$  is a peptide sequence, wherein each of  $AA^{1}$  through  $AA^{i}$  is an 29 amino acid residue which is a member independently selected from the 30 31 group of natural amino acid residues, unnatural amino acid residues and 32 modified amino acid residues; 33 J denotes the number of amino acid residues forming said peptide 34 sequence and is a member selected from the group consisting of 35 the numbers from 2 to 10, such that J-2 is the number of amino acid residues in the peptide sequence exclusive of AA<sup>1</sup>-AA<sup>2</sup>; and 36 i denotes the position of said amino acid residue in sequence relative to 37 38  $AA^1$  and when J is greater than 2, i is a member selected from the 39 group consisting of the numbers from 3 to 10. 36 (currently amended): The library of fluorogenic peptides according to claim .1

$$O \longrightarrow P^1$$

$$NHC(O)AA^1 - AA^2 - (AA^i)_{J-2}$$

$$R^3$$

35, wherein said first peptide and said second peptide have the structure:

4 ,

3

5

1

2

2

wherein:

c is a member selected from the group consisting of the numbers from 0 to 6.

37 (original): The library of fluorogenic peptides according to claim 36, wherein said first peptide and said second peptide have the structure:

Appl. No. 10/686,884 Amdt. dated March 9, 2005 Reply to Office Action of September 9, 2004

3

1

1 2

1

2

3

4

- 38 (currently amended): The library according to claim 35, wherein said 2 fluorogenic moiety of said first peptide and said fluorogenic moiety of said second peptide are different fluorogenic moieties. 3
  - 39 (currently amended): The library according to claim 35, wherein said first peptide sequence and said second peptide sequence are identical.
- 1 40 (currently amended): The library according to claim 35, wherein said first peptide sequence and said second peptide sequence are different. 2
  - 41 (currently amended): The library according to claim 40, wherein an amino acid residue selected from the group consisting of AA<sup>1</sup>, AA<sup>2</sup>, AA<sup>i</sup> and combinations thereof of said first peptide is a different amino acid residue than an amino acid residue at a corresponding position relative to AA<sup>1</sup> of said second peptide.
- 42 (currently amended): The library according to claim 35, wherein AA<sup>1</sup> of said 1 first peptide sequence and AA1 of said second peptide sequence are identical amino acid 2 residues. 3
- 43 (currently amended): The library according to claim 35, wherein AA<sup>1</sup> of said 1 first peptide sequence and AA1 of said second peptide sequence are different amino acid 2 residues. 3
- 44 (currently amended): The library according to claim 35, wherein AA<sup>2</sup> of said 1 first peptide sequence and AA2 of said second peptide sequence are identical amino acid 2 3 residues.

1	45 (currently amended): The library according to claim 35, wherein AA <sup>2</sup> of said
2	first peptide sequence and AA <sup>2</sup> of said second peptide sequence are different amino acid
3	residues.
1	46 (currently amended): The library according to claim 35, wherein AA <sup>i</sup> of said
2	first peptide sequence and AAi of said second peptide sequence are identical amino acid residues.
1	47 (currently amended): The library according to claim 35, wherein AA <sup>i</sup> of said
2	first peptide sequence and AA' of said second peptide sequence are different amino acid residues.
1	48 (original): The library according to claim 42, comprising at least six peptides
2	having different peptide sequences, wherein AA1 is a different amino acid residue in each of said
3	different peptide sequences.
1	49 (original): The library according to claim 48, comprising at least twelve
2	peptides having different peptide sequences wherein AA1 is a different amino acid residue in
3	each of said different peptide sequences.
1	50 (original): The library according to claim 49, comprising at least twenty
2	peptides having different peptide sequences wherein AA1 is a different amino acid residue in
3	each of said different peptide sequences.
1	51 (currently amended): The library according to claim 35, wherein AA <sup>1</sup> is a
2	member selected from the group consisting of Lys, Arg, Leu and combinations thereof.
1	52 (currently amended): The library according to claim 35, wherein $J$ is a
2	member selected from the numbers from 4 to 8.
1	53 (currently amended): The library of peptides according to claim 35, wherein
2.	at least one of said first peptide and said second peptide is cleavable by a protease into a
3	fluorescent moiety and the peptide sequence.

1	54 (currently amended): The library according to claim 55, comprising at least
2	10 peptides, wherein each of the peptide sequences is a different peptide sequence.
1	55 (original): The library according to claim 54, comprising at least 100
2	peptides, wherein each of the peptide sequences is a different peptide sequence.
1	56 (original): The library according to claim 55, comprising at least 1,000
2	peptides, wherein each of the peptide sequences is a different peptide sequence.
1	57 (original): The library according to claim 56, comprising at least 10,000
2	peptides, wherein each of the peptide sequences is a different peptide sequence.
1	58 (original): The library according to claim 57, comprising at least 100,000
2	peptides, wherein each of the peptide sequences is a different peptide sequence.
1	59 (original): The library according to claim 58 comprising at least 1,000,000
2	peptides, wherein each of the peptide sequences is a different peptide sequence.
1	60 (currently amended): The library according to claim 35, wherein said first
2	peptide is located at a first region of a substrate and said second peptide is located at a second
3	region of a substrate.
1 .	61 (currently amended): A method of determining a peptide sequence specificity
2	profile of an enzymatically active protease, said method comprising:
3	(a) contacting said protease with a library of peptides according to claim 35 in
4	such a manner whereby the fluorogenic moiety is released from the
5	peptide sequence, thereby forming a fluorescent moiety;
6	(b) detecting said fluorescent moiety;
7	(c) determining the sequence of said peptide sequence, thereby determining said
8	peptide sequence specificity profile of said protease.

I	62 (original): The method according to claim 61, further comprising (d)
2	quantifying said fluorescent moiety, thereby quantifying said protease.
1	63 (original): A database comprising at least one set of peptide sequence
2	specificity data for a protease determined using a library according to claim 35.
1	64 (original): The database according to claim 63, wherein said database is an
2	electronic database.
1	65 (original): The database according to claim 64, wherein said database is
2	distributed on a wide area network.
1	66 (original): A database comprising at least one set of peptide sequence
2	specificity data for a protease determined using a method according to claim 61.
1	67 (original): The database according to claim 63, wherein said database is an
2	electronic database.
1	68 (original): The database according to claim 64, wherein said database is
2	distributed on a wide area network.
1	69 (currently amended): The method according to claim 61, wherein said
2	protease is a member selected from the group consisting of aspartic protease, cysteine protease,
3	and serine protease.
1	70 (original): The method according to claim 61, wherein said protease is a
2	protease of a microorganism.
1	71 (original): The method according to claim 70, wherein said microorganism is
2	a member selected from the group consisting of bacteria, fungi, yeast, viruses, and protozoa.

Appl. No. 10/686,884 Amdt. dated March 9, 2005 Reply to Office Action of September 9, 2004

- 72 (original): The method according to claim 61, further comprising (c)
- 2 quantifying the fluorescent moiety, thereby quantifying said protease.

**73-83** (cancelled)